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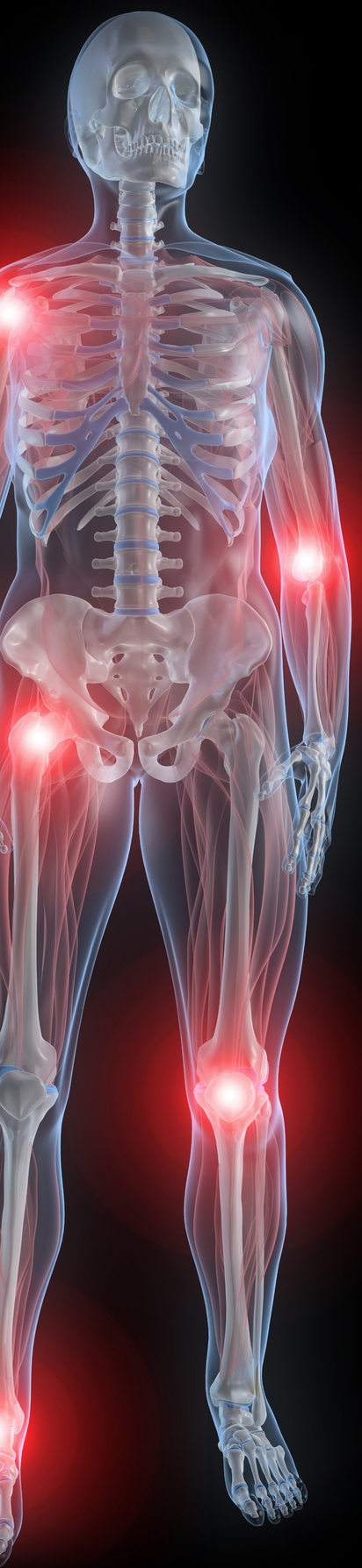

Long-term storage of clinical samples in CyMol® medium for PNA- FISH® and culturing from the eSwab™ system

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Objectives: The diversity of bacteria reported in foreign body infections is steadily growing, and culture-independent methods have become a valuable supplement to established culture methods. Therefore, sampling and preservation of specimens for molecular analysis have become an important issue. We report here experience gained with different specimen types obtained in parallel both pre- and peroperatively in patients enrolled prospectively in a clinical study of prosthesis-related problems (www.joint-prosthesis-infection-pain.dk).

Methods: Parallel sampling for both culture-dependent and -independent analyses were done over a period of two years. Specimens included tissue biopsies, bone biopsies, joint fluid, and eSwabs™ (Copan, Italy) taken from the prosthesis *in situ,* as well as sonication fluid (if prosthesis components were removed). Transfer to the laboratory was direct (ambient temperature) and storage temperature for culture specimens was +4°C (max 24 h), and -80°C for specimens for culture-independent methods (until batchwise analysis). Specimens for peptide nucleic acid-fluorescence *in situ* hybridization (PNA-FISH) analysis were stored in CyMol® medium (Copan, Italy) for up to one year. Direct visualization of microorganisms followed a previously published PNA-FISH® (AdvanDx, USA) protocol except for a substitution of the fixation step with filtration of 200 μL sample through a 0.22 μm white polycarbonate filter (prewashed with dH_2O, GE Water & Process Technologies, USA) in order to fix and concentrate the sample.

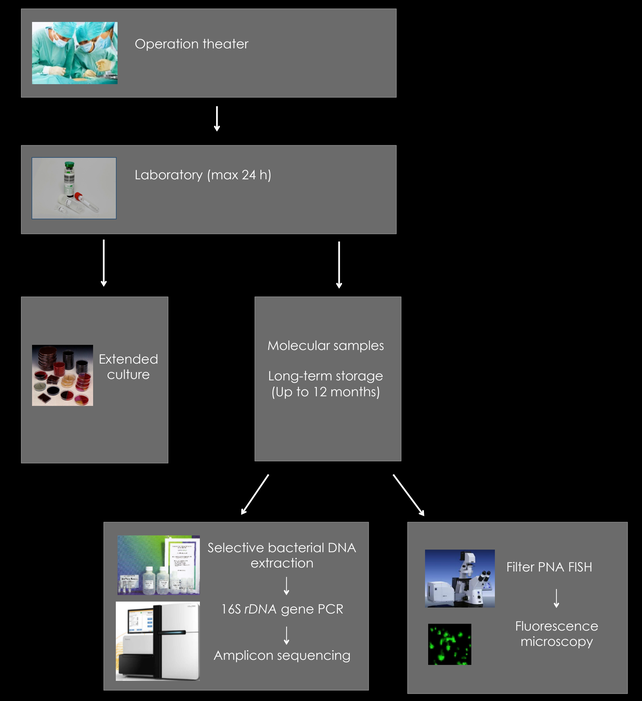
Results: With broad range and specific PNA-FISH® probes, we demonstrated bacteria with a bright signal and morphology comparable to the isolates obtained by culture of parallel specimens within 24 h. The detection limit for PNA-FISH® were >10^2 CFU/mL (estimated from the colony forming units cultured, updated from abstract). With the eSwab™ system we detected a broad range of Gram-positive taxa including *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., and *Corynebacterium* spp. by culture and 16S rRNA gene amplicon sequencing (table 1).

Conclusion: The use of the CyMol® medium made it possible to preserve samples at -80°C for study by PNA-FISH® for at least 12 months and we expect storage for even longer periods to be feasible. We estimated the effective detection limit to be in the order of >10^2 CFU/mL (updated from the abstract). Both the morphology and intensity of staining with nucleic acid and PNA probes were distinct. The eSwab™ was a convenient system for documenting a broad range of bacterial pathogens associated with foreign body infections.

	Direct culturing	**16S *rDNA* sequencing**
	eSwab™ in modified Amies medium	eSwab™ stored in modified Amies medium with 20% glycerol at -80 °C
Staphylococcus aureus	+	+
Staphylococcus epidermidis	+	+
Staphylococcus lugdunensis	+	+
Staphylococcus caprae	+	+
Enterococcus faecalis	+	
Streptococcus dysgalactiae	+	+
Streptococcus agalactiae	+	+
Propionibacterium acnes	+	+
Corynebacterium jeikeium	+	+
Corynebacterium striatum	+	
Escherichia coli		+

Table 1: Bacterial species recovered by eSwab™ (Copan, Italia). eSwabs™ used for culturing were transported in modified Amies medium and cultured within 24 h. eSwabs™ for sequencing were stored at -80 °C in modified Amies medium with 20% glycerol until analysed batchwise.

Flow diagram



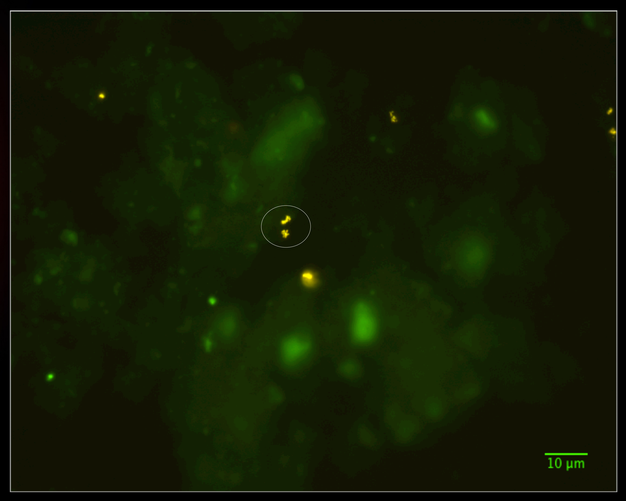


Figure 1: Filter PNA-FISH® on sonication fluid from hip prosthetic component. Universal bacterial probe (BacUni; green, *Enterococcus faecalis*; red. The culture and sequencing were positive for *E. faecalis*. The PNA-FISH confirmed the culturing and sequencing results illustrating yellow bacteria in clusters (eg. circled).

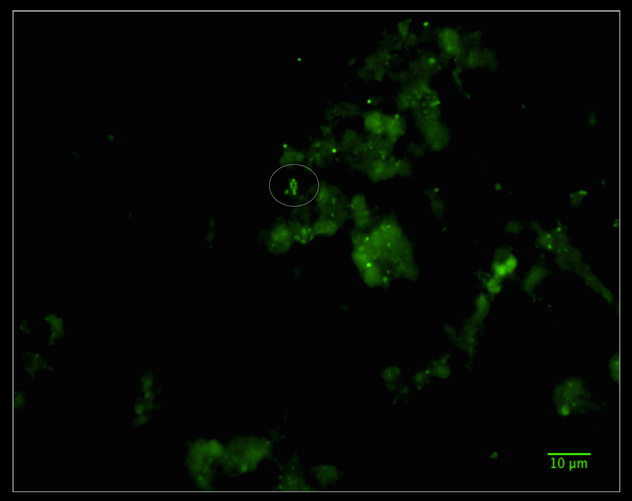


Figure 2: Filter PNA-FISH® on sonication fluid from knee prosthetic component. *Staphylococcus aureus*; green. The culture and sequencing were positive for *S. aureus*. The PNA-FISH confirmed the culturing and sequencing results illustrating green bacteria in clusters (eg. circled).

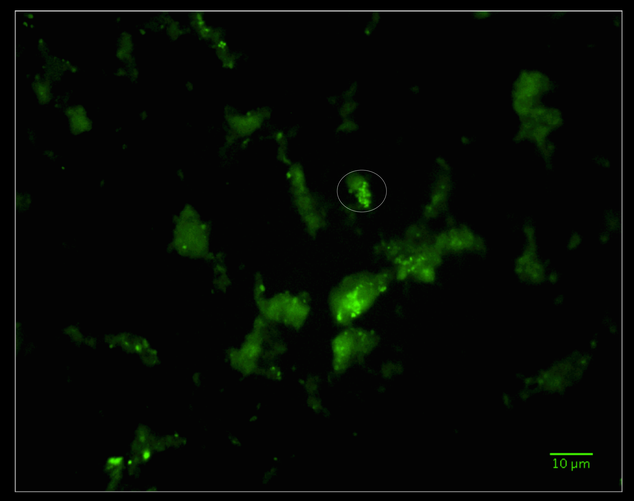


Figure 3: Filter PNA-FISH® on sonication fluid from knee prosthetic component (same case as figure 2). *Staphylococcus aureus*; green. The culture and sequencing were positive for *S. aureus*. The PNA-FISH confirmed the culturing and sequencing results illustrating green bacteria in clusters (eg. circled).

Acknowledgement
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